A SELF-CLEANING, CONTINUOUS COOLING BELT FOR LOW TEMPERATURE MOLECULAR LUMINESCENCE SPECTROMETRY

By

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Abstract of Dissertation Presented to the Graduate School
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A SELF-CLEANING, CONTINUOUS COOLING BELT
FOR LOW TEMPERATURE MOLECULAR LUMINESCENCE SPECTROMETRY

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Chairman: James D. Winefordner
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Sample cooling devices often improve the sensitivity and selectivity of measurements in optical spectroscopic methods for chemical analysis. Such devices are commonly used for matrix isolation infrared absorption and Raman spectrometry, ultraviolet absorption spectrometry, and molecular luminescence spectrometry. This work describes a device for rapidly cooling small amounts of sample to 15 K. The device employs a thin brass belt connected at the ends to form a loop. At one end, the loop is in contact with the cold stage of a closed-cycle helium refrigerator. Fifty microliter volumes of samples are injected through a septum in the vacuum chamber onto the cold portion of the belt. After the analysis, the belt carries the sample away from the cold stage where it warms and sublimes. The cooling device is therefore self-cleaning. Eleven polycyclic aromatic hydrocarbons (PAHs) dissolved in

Shpol'skii solvents are determined by xenon arc excited low temperature molecular luminescence spectrometry (LT-MLS). Subsequently, the sample cooling device is used for the determination of several PAHs in cooked beef at the part per billion level. Commercial and "home-grilled" hamburgers are extracted with a Shpol'skii solvent and injected onto the belt. Results are compared with those obtained by constant energy synchronous luminescence spectrometry (CESLS) at 77 K and by high performance liquid chromatography (HPLC) with multiwavelength detection.

CHAPTER 1

LOW TEMPERATURE MOLECULAR LUMINESCENCE SPECTROMETRY

Introduction

Cooling samples to liquid nitrogen temperature and below has been shown to increase the sensitivity of analytical methods involving molecular luminescence (1). Unfortunately, even at liquid helium temperature, the luminescence spectrum of most organic compounds consists of a few broad bands (100-1000 cm^{-1}). The causes for this band broadening fall under two categories: homogeneous broadening and inhomogeneous broadening. Homogeneous broadening is the result of several band broadening mechanisms such as rotational and vibrational broadening. Rotational broadening arises because large molecules have minuscule energy spacings between rotational levels. At a given temperature, many different rotational levels may be populated in the ground state and several rotational levels in the first excited state may be populated by optical excitation. As a result, each vibronic absorption or fluorescence band exhibits a rotational contour rather than a discrete line (2). Similarly at ambient temperatures and higher, more than one vibrational level may be excited in the ground state. Absorption spectra in the gas phase are thus vibrationally broadened. Both of these sources of broadening are temperature dependent and are greatly reduced in

frozen sample matrices. At low temperatures purely homogeneous broadening would lead to fluorescence band widths less than 5 cm^{-1}.

By far the most important source of band broadening in molecular luminescence spectrometry is inhomogeneous in nature. Purely inhomogeneously broadened spectra have a rich latent fine structure. In the region of each vibronic transition of a single molecule, the spectrum contains a narrow zero phonon line (<5 cm^{-1}), and in some cases a less intense phonon wing (<100 cm^{-1}) (Figure 1). However, differences in the local microenvironment for individual molecules result in a statistical scatter in the positions of their spectral bands. The overall spectral band appears as the smeared sum of these individual bands (3).

Before low temperature molecular luminescence spectrometry can be used as a method for the qualitative determination of individual components of a complex mixture, this inhomogeneous band broadening must be greatly reduced or eliminated. Several techniques have been developed to deal with this problem. Some of the most successful of these techniques are site-selection spectrometry, supersonic jet spectrometry, matrix isolation spectrometry and Shpol'skii spectrometry.

In site-selection spectrometry, the liquid sample is frozen to temperatures below 40 K so that it forms an amorphous glass. Inhomogeneous broadening is eliminated by selective laser excitation in the appropriate region of the absorption spectrum of the analyte. Mainly those molecules having a zero phonon line at the laser frequency are excited. Only the narrow fluorescence lines belonging to those molecules are observed in the spectrum (4). An obvious

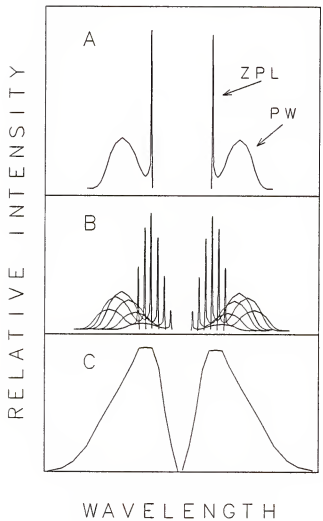

Figure 1. Scheme for the formation of inhomogeneously broadened spectral bands. A) Absorption (left) and emission bands of a single molecule showing the zero phonon line (ZPL) and the phonon wing (PW); B) The individual spectral bands of several molecules occupying six different sites in the matrix. C) the observed spectral bands.

drawback to this technique is the inherent loss of sensitivity. A high percentage of analyte molecules are not excited by the laser because they have slightly different microenvironments. Nevertheless, part-per-billion levels of detection have been reported for several polycyclic aromatic hydrocarbons (PAHs) in organic glasses (5). The technique has also been applied to the determination of PAHs in real samples such as solvent-refined coal (6).

A more practical approach to reducing inhomogeneous broadening is to find a matrix in which all analyte molecules have similar microenvironments. In such a case, no highly selective excitation step is required. One such technique involves expanding the sample in a supersonic jet. A gaseous sample at ambient pressure and temperature may be expanded through a small orifice (<1 mm) into a region of high vacuum (<1 mTorr). Near the orifice, the sample molecules are rotationally and vibrationally cooled through molecular collisions via the Joule-Thomson effect. Temperatures less than 5 K may be observed (7). At some point further downstream, the molecular collision frequency becomes negligible since the gas is expanding in a vacuum and the supersonic beam enters the free molecular flow regime (2). Since no analyte molecule is in contact with any other molecule, the microenvironments are identical. Inhomogeneous broadening is eliminated and narrow line fluorescence results even with broad band excitation (8-10). Advantages of this technique include elimination of solvent effects, extremely low sample temperatures without liquid coolants or refrigerators, and applicability to a wide variety of analyte molecules (11).

Disadvantages include loss of sample during the measurement, the necessity of converting all samples into the gaseous state, and low sensitivity due to the huge sample dilution in the vacuum chamber (12).

Another technique used to produce similar microenvironments for each analyte molecule is matrix isolation spectrometry (13). In this technique, a sample gas is diluted in a matrix gas and frozen onto a cold surface (<50 K) in a vacuum. The matrix gas may be argon, nitrogen, an n-alkane, or some other such solvent. In the ideal case, narrow line fluorescence is observed with broad band excitation. More often, however, selective laser excitation is used. The main advantage of this technique is that the analyte need not be soluble in the matrix gas or liquid since the two are mixed and frozen simultaneously. Disadvantages include the necessity for producing the sample in a gaseous form, and the need for selective excitation much of the time. Matrix isolation fluorescence spectrometry has been applied to the determination of PAHs in solvent-refined coal (14). Matrix isolation in general has been applied to other spectroscopic techniques such as infrared absorption spectrometry (15).

By far the most common technique for the reduction of inhomogeneous broadening in fluorescence spectrometry is the use of the well-known Shpol'skii effect as an analytical tool in the determination of PAHs (16). The following section is devoted to a detailed discussion of the Shpol'skii effect.

The Shpol'skii Effect

In 1952, Soviet physicist E. V. Shpol'skii and coworkers published a paper entitled "Fluorescence Spectrum of Coronene in Frozen Solutions" (17). They discovered that "an astonishing change in spectrum was observed when n-hexane and n-heptane were used" as solvents. Instead of a small number of broad fluorescence bands, they observed "a large number of narrow lines which by their width are similar to lines of atomic spectra in gases" (p. 935). This effect, later called the Shpol'skii effect, was extensively studied in the later 1950s and several papers were devoted to understanding the phenomenon. In 1960, Shpol'skii published a review entitled "Line Fluorescence Spectra of Organic Compounds and Their Applications" (18). The narrow line fluorescence of PAHs in frozen Shpol'skii matrices was later attributed to dimensional and geometrical considerations between aromatic solute molecules and their n-alkane hosts (19,20). This so-called "lock and key" principle is still accepted today and several recent publications have described the effects of alkyl chain length on Shpol'skii spectra (21,22). A graphic representation of the Shpol'skii effect is shown in Figure 2. The broad band fluorescence spectrum of perylene in ethanol is reduced to a quasi-linear spectrum in the Shpol'skii solvent. Due to the quasi-linear spectra that they exhibit, PAHs have been qualitatively identified by Shpol'skii fluorescence spectrometry in samples such as aquatic sediments, crude oils and liquid fuels (23-26). A nearly comprehensive atlas of Shpol'skii spectra has been published in this decade (27).

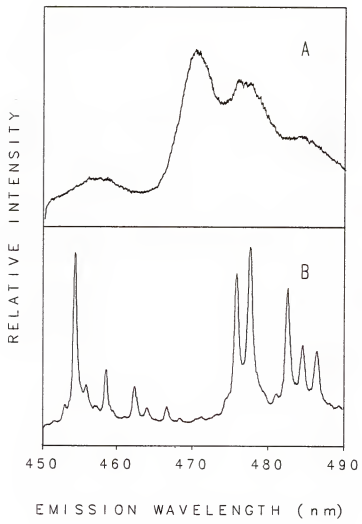

Figure 2. The fluorescence spectrum of perylene (700 ng/mL) in two different solvents. A) ethanol; B) hexane.

The use of the Shpol'skii effect in the quantitative determination of PAHs has developed slowly over the past twenty years. At first the combined instrumental and theoretical restrictions seemed to limit the usefulness of true quasi-line spectra for analytical determinations (28). Later, improved techniques made quantitative applications more feasible (29). Since then, the Shpol'skii effect has become a useful tool in analytical spectrometry. Analytical figures of merit have been recorded in the literature and the analysis of complex mixtures is possible with little or no sample preparation (30,31). The technique has been applied to the identification and quantification of PAHs in environmental samples such as coal liquids (32), automobile exhaust (33), and air-borne particulates (34). A good review on the analytical utility of Shpol'skii spectrometry has been published recently (16).

The use of the Shpol'skii effect has several advantages over the other techniques used for the reduction of inhomogeneous band broadening: samples need not be present in the gaseous form, the frozen sample matrix is easily prepared, and no selective excitation is required although laser excitation is used in many cases (35). The technique does have some disadvantages. The analyte must be soluble in the n-alkane solvent. Also, the technique is limited to the determination of PAHs and some of their derivatives. Only these molecules have a suitable geometrical configuration to align themselves with the Shpol'skii matrix.

The Effect of Lowering Sample Temperature

As stated earlier, lowering the temperature of the sample decreases homogeneous broadening in molecular luminescence spectra. If the sample changes from a liquid to a solid matrix, inhomogeneous broadening may also be decreased in special cases like those involving the Shpol'skii effect. This reduction in fluorescence band widths is demonstrated in Figure 3. In the transition from 298 K to 77 K, the Shpol'skii effect is observed for perylene in hexane since the sample freezes. Upon further reduction in sample temperature to 15 K, additional decreases in homogeneous broadening are observed. This effect has been reported by several research groups (36,37).

Another interesting consequence of lowering the sample temperature is the increased intensity of the fluorescence bands. This effect, not obvious in Figure 3, is demonstrated by Figure 4. At lower temperatures the motion of the analyte molecules is reduced. This decreases the rate of collisional deactivation of the first excited electronic state of the molecule. As a result, the quantum efficiency for fluorescence is increased and a corresponding increase in fluorescence intensity is observed. Reduction in the rate of collisional deactivation also increases the lifetime of the first excited state, thus making the observance of phosphorescence more probable. In fact, phosphorescence is often observed in frozen matrices but only rarely occurs at ambient temperatures.

Sample Cooling Techniques

A major consideration encountered in the design of low temperature luminescence systems is the choice of sample cooling

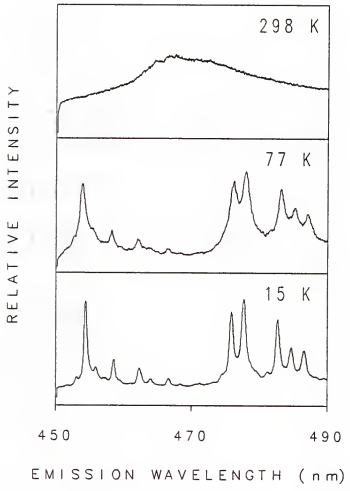

Figure 3. The fluorescence spectrum of perylene (700 ng/mL) at three different temperatures.

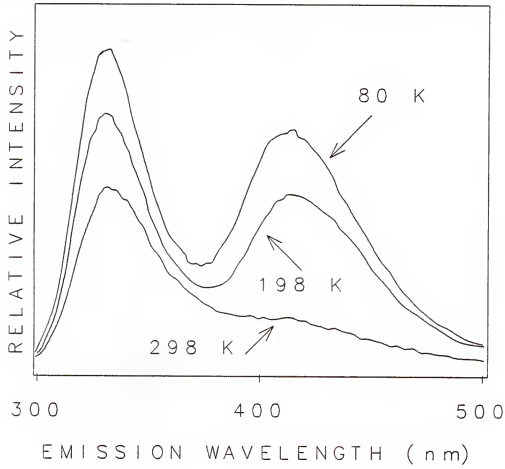

Figure 4. The luminescence spectrum of p-aminobenzoic acid at three different temperatures (37).

device. Three methods are commonly used for such cooling. A liquid sample held in a quartz tube cell may be immersed in a Dewar flask filled with a coolant such as liquid nitrogen or liquid helium (29-31). A similar sample may be cooled by conduction from a metallic rod immersed in the liquid coolant (33, 38-40). The sample may also be cooled while it is in contact with the cold stage of a closed-cycle Joule-Thomson helium refrigerator (41-43). Each method has advantages and disadvantages. Immersion cooling is fast and readily adaptable to commercial fluorescence instruments. Most companies sell a liquid nitrogen sample cooling arrangement that fits into the sample compartment of their instrument. On the other hand, the handling of liquid nitrogen or liquid helium can be expensive, time consuming and tedious, involving the manipulation of cumbersome Dewar flasks. Another problem may also be scattered source radiation from all of the interfaces present in the optical pathways with the Dewar flasks and sample tube. Conduction cooling reduces this problem since the sample is not immersed in the liquid nitrogen. However, since the cold sample is open to the atmosphere, care must be taken that water vapor is not allowed to freeze onto the sample surface. This is usually accomplished by flushing the sample with dry nitrogen gas during the measurement process. Joule-Thomson refrigerators eliminate the need for liquid coolants. They also reach temperatures well below that of liquid nitrogen and can be tuned to a specific temperature with a small, built-in heater. Unfortunately, these refrigerators are usually large and bulky, often requiring elaborate vacuum systems and expensive gas compressors. Nevertheless, the

elimination of the liquid coolant makes these devices the most attractive sample cooling systems.

Another important disadvantage with closed-cycle refrigerators is the long cool down time associated with them. On the average, these refrigerators can cool a sample holding device from room temperature to 15 K in about one hour (41). Certainly such a long cool down time is impractical analytically if each sample must be cooled separately. As a result, several attempts have been made to deposit more than one sample onto the cold stage at a time. When this is done, each sample must be moved individually into the viewing region of the spectrometric system. Therefore, some sort of moving sample cooling device must be employed.

Moving Sample Cooling Devices

The simplest moving sample cooling device is one similar to that used by Garrigues (44). A stationary copper cold stage is fitted with five to ten compartments for holding small quartz tubes filled with sample (Figure 5). The samples are cooled simultaneously under vacuum to a temperature near 15 K. Each sample is placed in the viewing position of the spectrometric system by moving the entire refrigeration system in a horizontal direction. After the analyses, the refrigerator is warmed and the samples are changed. The advantage of this set-up is that sample changes are simple, requiring no special sample introduction procedures. The vacuum chamber is opened and the samples are manually attached to the cold stage. The disadvantages include the limited sample capacity of the cold stage, resulting in increased time required for analysis. Also, the entire

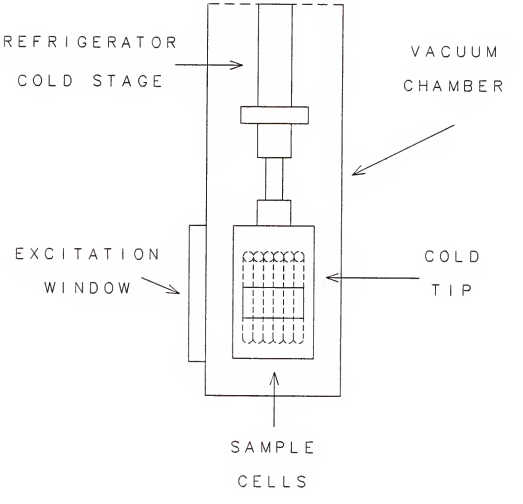

Figure 5. Sample cooling device designed by Garrigues et al. Redrawn from figure in reference 44.

cryostat must be moved. Accurate alignment of each sample may be difficult due to the bulky size of the system.

A more sophisticated moving sample cooling device was introduced in 1979 (45). It differs from previous devices since it permits the cryogenic surface on which the samples are held to be moved independently of the adjoining apparatus including the vacuum chamber and the closed-cycle refrigerator (Figure 6). This allows positioning of the frozen samples for subsequent spectroscopic examination. The cold surface is an eight-sided carousel threaded at the central core. The internal threads of the carousel engage with a stationary threaded shaft anchored to the cold stage of the cryostat. The carousel is moved by a pinion gear that is coupled to a spur gear at the top of the carousel. The pinion gear is turned via a rotary motion vacuum feedthrough. By raising or lowering the entire cryostat, four different vertical levels may be selected so a total of 32 samples may be cooled at once. After the analyses, the cooling device must be heated and cleaned.

A third sample cooling device also employs a cold sample holder that moves relative to the vacuum chamber and cryostat (46,47). The twelve-sided disk is turned by a rotary motion vacuum feedthrough connected to it by an insulated spacer (Figure 7). In this arrangement no pinion gears are necessary. After all of the sample positions have been used, the refrigerator must be warmed for cleaning.

In the fourth sample cooling device, the sample holder is rotated relative to the cold stage (42). The sample holder is a hollow cylindrical metal block physically attached to the cold stage

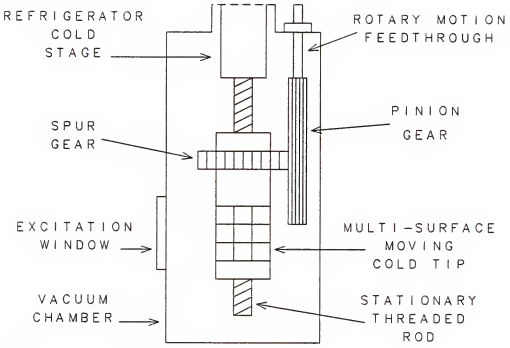

Figure 6. Moving sample cooling device designed by Reedy et al. Redrawn from a figure in reference 45.

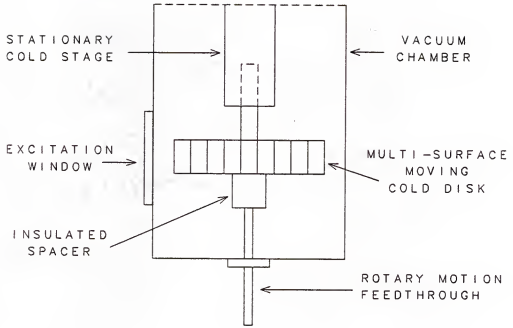

Figure 7. Moving sample cooling device designed by Wehry et al. Redrawn from a figure in reference 47.

of the refrigerator by a flexible, multileaf copper coil (Figure 8). Sixty sample compartments are positioned around the cylinder in five rows. A particular row is chosen by moving the entire cryostat in the vertical direction. The horizontal position is chosen by turning the sample holder relative to the cold stage via a rotary motion vacuum feedthrough. After the sixty samples have been analyzed, the cold stage must be heated to remove them.

With the final moving sample cooling device, the sample is frozen onto a cold metal disk firmly bolted to the refrigerator cold stage (41,48-51). A double o-ring seal between the base of the refrigerator and the vacuum shroud allows the entire cryostat to rotate independently of the vacuum chamber (Figure 9). The sample, in the form of effluent from a gas chromatography column, is frozen onto the disk as it rotates. Three complete revolutions of the cryostat may be achieved, providing six hours of continuous sampling time. After this time, the cold stage must be heated to remove the samples collected and the entire sample collection process can be repeated. This has been the most successful moving sample cooling device to date and it is now available commercially. Its major advantages over the other devices are that it samples continuously rather than in small discrete segments and that it allows for longer sample collection times.

Although these sample moving techniques are very useful analytically, they suffer from several drawbacks. Most of the devices require movement of the entire cryostat at some point. This is cumbersome since the refrigerator is quite heavy with several electrical connections and high pressure gas lines attached.

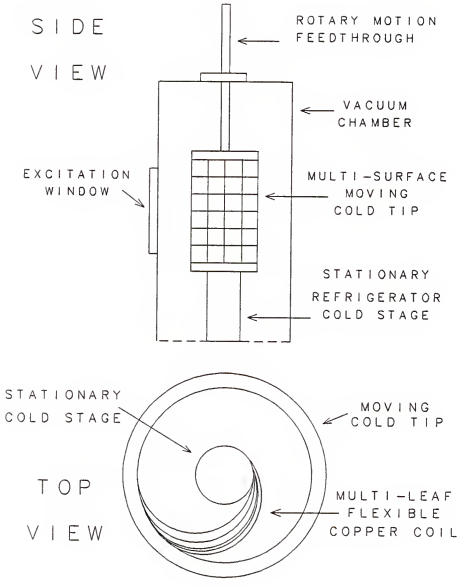

Figure 8. Moving sample cooling device designed by Margrave et al. Redrawn from a figure in reference 42.

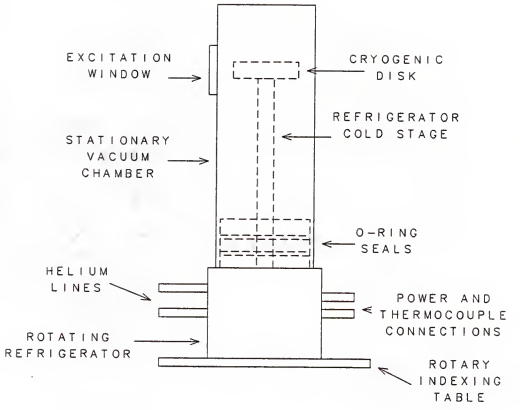

Figure 9. Moving sample cooling device designed by Reedy et al. Redrawn from a figure in reference 41.

Accurate positioning of the cryostat may require expensive and elaborate mechanical devices. Most of the sample moving devices allow sample collection only at a certain designated positions on the sample holder (only the last device described allows continuous sampling). All of the devices provide only limited sampling time between refrigerator down time. Finally, none of the devices are self-cleaning. In each case, the cryostat must be heated to room temperature to remove the samples.

The next chapter presents a self-cleaning, continuous cooling belt for low temperature molecular luminescence spectrometry. The newly constructed device eliminates all of the drawbacks mentioned above. Since the device is self-cleaning, it may be operated indefinitely at low temperature without the need for warm-up. Also, the device is constructed so that movement of the entire cryostat is unnecessary.

CHAPTER 2

A SELF-CLEANING, MOVING SAMPLE COOLING DEVICE

Introduction

Using the five devices discussed earlier as a basis, the goal of this work was to design and construct an improved moving sample cooling device. The ideal cooling system should meet the following criteria:

1) the device will quickly cool a sample to 15 K,
2) the device can be operated indefinitely without the need for refrigerator down time,
3) the device can accommodate samples continuously or in discrete segments,
4) the device is self-cleaning,
5) the device does not require the movement of the entire cryostat,
6) the device has only a limited number of small moving parts,
7) the device is easy to construct and to operate,
8) the device is relatively inexpensive to build.

None of the cooling systems described in Chapter 1 meet all of these criteria. In fact, none of those systems meet half of them. In the next section, a sample refrigeration system is described that satisfies all of these requirements.

Design and Construction

The sample cooling cryostat is the commercially available Displex Model CS-202 by Air Products Co. (Allentown, PA). It is a closed-cycle helium refrigerator that cools a copper cold tip to 11 K in 45 minutes without the need for liquid helium. The cold tip was laboratory-designed and is made of oxygen-free high conductivity copper (Figure 10). It consists of two parts: a horseshoe-shaped frame that is bolted to the cold stage by a 1/4-28 copper bolt and a stationary spool held in the frame by a 1/4-20 copper bolt with nut. The spool dimensions are 1/2 in wide with a 3/4 in diameter. A groove is cut around the spool that is 1/64 in deep and 3/4 in wide. The dimensions of the frame are 1 1/2 in x 3/4 in x 3/4 in.

A brass belt 13 in long and 1/4 in wide is cut from a piece of 0.002 in thick stock. The ends of the brass belt were spot-welded together so that the belt forms a continuous loop. The belt is threaded around the copper spool so that it fits in the groove. The other end of the belt is threaded around a plastic spool attached to a rotary motion vacuum feedthrough. The two spools are spaced so that the brass belt is taut (Figure 11). The groove in the plastic spool is lined with rubber to supply adequate friction to slide the belt across the copper spool.

The vacuum chamber itself is also laboratory-designed. It is constructed from a piece of 3 in o.d. stainless steel pipe, flanges, and a hollow stainless steel block (Figure 12). The bottom of the pipe is attached to the base of the refrigerator by a double o-ring seal. The top of the pipe is welded to a cap fitted with a septum port in the middle. The septum port is a stainless steel 1/4 in

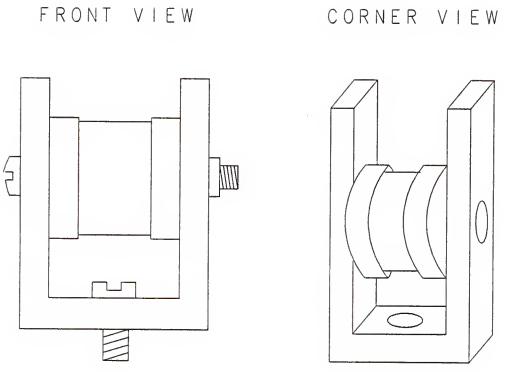

Figure 10. Detailed drawing of the copper cold tip.

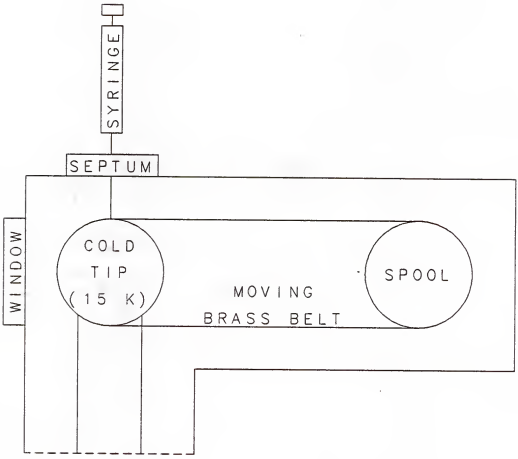

Figure 11. Simplified cross-sectional view of the upper portion of the vacuum chamber.

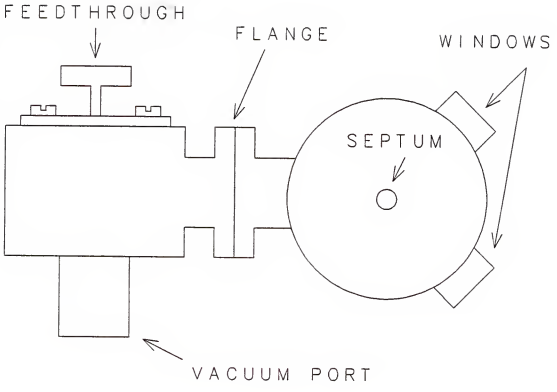

Figure 12. Top view of the vacuum chamber.

Cajon fitting and is positioned directly over the cold tip. The septum is a 3/8 in high-temperature gas chromatography septum from Alltech Associates, Inc. (Deerfield, IL). On the sides of the tube, at the height of the cold tip, are two viewing window ports positioned 90° apart from one another. The window ports are also Cajon fittings holding 1 in round quartz windows with o-ring seals. Positioned 135° from each window is a port connected to a 2 3/4 in flange. The flange connects the vacuum chamber to a hollow block that houses the rotating spool. The block is fitted with a 2 3/4 in port to the vacuum pump. The vacuum pump connected to the sample chamber ia a Leybold Heraus Trivac-A dual stage roughing pump (Export, PA). Also fitted to the block is a face plate attached with four screws and sealed with an o-ring. In the center of the face plate is a 1 in diameter hole used for mounting the rotary motion vacuum feedthrough (Huntington Mechanical Labs, Mountain View, CA). The plastic spool is mounted on the vacuum end of the feedthrough and a hand-turned knob is mounted on the outside end (Figure 13). The entire face plate can be moved right or left about 1/4 in to ensure that the brass loop is tight.

Operation

After an initial cool down period, the cold tip is kept at 15 K or below until completion of the experiment. Pressure inside the chamber is maintained at 3 mTorr. Sample injections are made as follows. The excitation monochromator is set to a visible wavelength so the cold tip is well illuminated through one of the windows. A small dental mirror is used for viewing inside the vacuum chamber

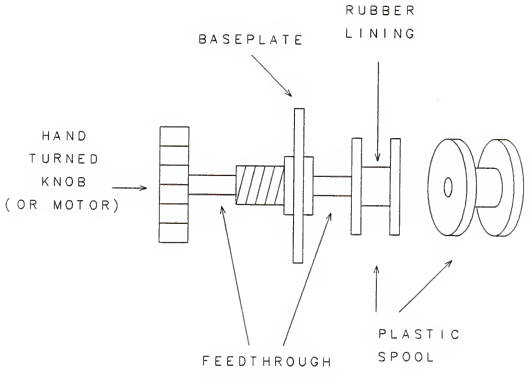

Figure 13. Schematic diagram of the moving plastic spool and rotary motion vacuum feedthrough.

through the other window. A disposable, 1.0 mL syringe with a 3 in. needle is filled to the 50 μL mark. The needle is passed through the septum until it rests against the brass belt in contact with the cold tip (as in Figure 11). The sample is injected slowly so that a single 50 μL drop forms on the belt. An initial rise in temperature is noticed on the cold tip, usually to about 25 or 30 K. After about two minutes, the cold tip reaches a temperature below 15 K. Next the belt is moved via the rotary motion feedthrough so that the excitation light focused onto the cold tip covers the sample. Once the sample is in place, the appropriate spectral measurement is performed. After the spectrum is stored, the band is moved so that the portion with the sample is no longer in contact with the cold tip. The sample quickly warms, evaporates and is pumped away. The cooling device is therefore self-cleaning. For continuous measurements, the rotary motion feedthrough is turned by a stepping motor. A flowing stream of gas or liquid is frozen directly onto the continuously moving belt.

CHAPTER 3

ANALYSIS OF SAMPLES CONTAINING POLYCYCLIC AROMATIC HYDROCARBONS

Introduction

Polycyclic aromatic hydrocarbons (PAHs) make up a very important group of chemical compounds some of which are potent carcinogens (52). Also called polynuclear aromatic hydrocarbons, they can be defined as organic compounds containing two or more fused benzene rings which may have substituent groups attached to one or more of the rings. More than 100 different PAHs have been identified in the environment.

Polycyclic aromatic hydrocarbons would be present in the environment even without the intervention of man. Geochemical sources include coal, minerals, volcanic activity and sedimentary rock. They may also arise from biochemical synthesis in plants and bacteria, or as products from naturally occurring forest fires (53). The relatively low levels of PAHs from these sources is dwarfed by the amount arising from anthropogenic sources however. Aromatic compounds are known to be produced by the pyrolysis of hydrocarbons, such as fossil fuels (54). The United States alone pumps an estimated 1320 tons of 3,4-benzopyrene into the atmosphere each year (55). Of that amount, 500 tons is produced by power generation, 600 tons by refuse burning, 200 tons from coke production and 20 tons from motor vehicles. The total amount of PAHs produced world-wide

probably exceeds 50,000 tons per year. Obviously, these compounds are distributed throughout the multimedia environment. They have been identified in lakes and rivers (56,57), river sediments (24), soils and plant systems (53), and foods (58-60).

Due to their abundance, total removal of PAHs from the environment is nearly impossible. Their effects on human health justify that levels in food and drinking water be closely monitored and regulated if possible. In 1915, coal tar painted on the ears of rabbits and mice was found to promote the development of tumors after repeated applications (59). Later some isolated compounds, including 3,4-benzopyrene, present in the coal tar were identified as carcinogens (61). Since these earlier studies, many other PAHs have been shown to have carcinogenic potential in animals. A total of 20 PAHs have been recognized as carcinogens by the Environmental Protection Agency (62) and by the National Academy of Sciences (55). Only 11 of these have been identified in foodstuffs. Out of these 11, only five (3,4-benzopyrene, 1,2-benzanthracene, 3-methylcholanthrene, 1,2-5,6-dibenzanthracene and 9,10-dimethyl-1,2-benzanthracene) have been demonstrated to induce tumors following oral administration to rats and mice. The cancers induced orally are restricted mainly to leukemia, forestomach tumors, hepatoma, and pulmonary adenoma. Insufficient evidence is available to determine whether these same PAHs induce similar cancers in humans.

The high carcinogenic effect of microgram quantities of these PAHs demands strict surveillance through highly sensitive and selective methods for their detection and determination in foodstuffs and in other materials with which human beings come into frequent

contact. Several analytical methods which have been developed or adapted for their isolation, identification and quantification (63) include liquid chromatography (64), gas chromatography (65), and fluorescence techniques such as Shpol'skii spectrometry (66). The next section describes the experimental arrangement used in this work for the detection of eleven PAHs by low temperature molecular luminescence spectrometry using a moving sample cooling device (67).

Experimental

Spectrometric system. The spectrometric system was constructed from commercially available components. A block diagram of the system is given in Figure 14. The sample cooling device is the one described in Chapter 2. The light source is a 150 W xenon arc lamp (Varian Eimac, Palo Alto, CA). White light is passed through a Jobin-Yvon model H.10 monochromator (Instruments SA, Metuchen, NJ) to select an excitation wavelength. Exciting radiation with a bandpass of 8 nm leaving the monochromator is focused onto the brass belt in contact with the cold tip. Fluorescence emitted from the analyte on the belt is collected by a lens and focused onto the entrance slit of a 0.5 m Spex (Metuchen, NJ) model 1870 Czerny-Turner Spectrograph with a bandpass of 0.4 nm. Radiation flux at the exit port of the emission monochromator is detected by a Princeton Instruments Model IRY-10246 Optical Spectrometric Multichannel Analyzer (OSMA) (Trenton, NJ). Similar detectors have previously been used for high resolution Shpol'skii spectrometry (68). The OSMA is a linear photodiode array 2.56 mm long with 1024 elements. It is water cooled

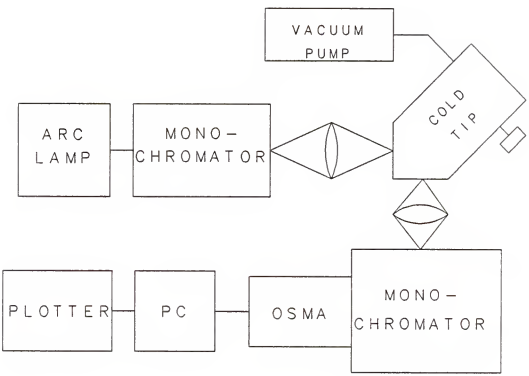

Figure 14. Block diagram of the spectrometric system.

to -24°C and is positioned at the exit focal plane of the spectrograph. The array length corresponds to 40 nm in wavelength units. The detector is interfaced with and controlled by a PC's Limited 286 series personal computer (Austin, TX). Spectra are plotted by a Hewlett Packard Model 7440A Color Pro Graphics Plotter (San Diego, CA).

Reagents. Table I is a list of all of the chemicals used in this study. All PAHs were used as received, without further purification. Stock solutions for each PAH were prepared in hexane with a concentration in the range of 20-100 mg/L, depending on the solubility of the particular compound. The stock solutions were diluted as necessary to cover a concentration range of four orders of magnitude.

Procedure. Refrigerator cool down and sample injections were accomplished as described in Chapter 2. Low temperature Shpol'skii spectra were stored for each PAH (see Appendix). At least three injections were made of each solution, and the three were averaged to use in preparation of analytical calibration curves. Calibration curves were plotted as peak height versus concentration.

Results and Discussion

Shpol'skii spectra. Quasi-linear Shpol'skii luminescence spectra were obtained for each of the eleven PAHs tested (see Figure 15 for example). The full-width at half-maximum (FWHM) was found for each compound at a particular emission wavelength. When possible, this emission wavelength was chosen in a region with little or no overlap of peaks. For convenience, the solvent employed in each case

Table I. List of PAHs and Solvents.

Chemical	Company	Address	Purity (%)
Anthracene	Mallinckrodt	Paris, KY	95
1,12-Benzoperylene	Aldrich	Milwaukee, WI	98
1,2-Benzopyrene	Aldrich	Milwaukee, WI	99
3,4-Benzopyrene	Aldrich	Milwaukee, WI	98
Coronene	Fluka	Ronkonkoma, NY	97
Naphthacene	Eastman	Rochester, NY	98
Naphthalene	Fisher	Fair Lawn, NJ	95
Perylene	Fluka	Ronkonkoma, NY	99
Phenanthrene	Eastman	Rochester, NY	98
Pyrene	Aldrich	Milwaukee, WI	99
Triphenylene	Aldrich	Milwaukee, WI	98
Hexane	Burdick & Jackson	Muskegon, MI	UV
Ethanol	Florida Distilleries	Lake Alfred, FL	100

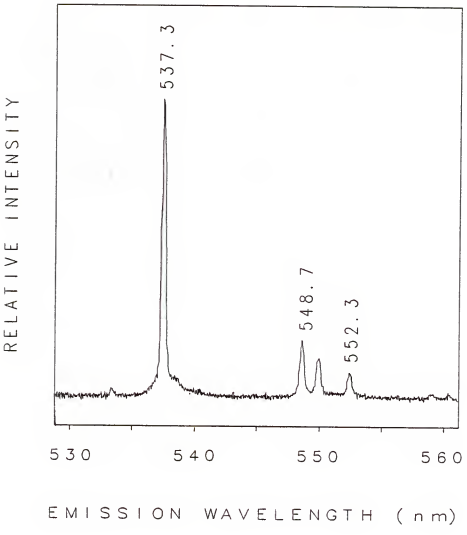

Figure 15. Phosphorescence spectrum of 1,2-benzopyrene at 15 K (Concentration 3 mg/L in n-hexane).

was hexane. Table II compares present results with those found in the literature at a higher temperature (31).

Analytical figures of merit. Analytical calibration curves were plotted for each compound (Figures 16-18). The emission wavelength used for each calibration curve corresponds to that peak in each spectrum giving the highest signal-to-noise ratio and, therefore, the best detection limit. Since the limiting source of noise in each case is scattered excitation radiation, phosphorescence peaks often have the highest signal-to-noise ratio due to the increased separation between excitation and emission wavelengths. Analytical figures of merit were determined from the curves and are reported in Tables III-V. Limits of detection (LODs) compare well with those in the literature at other temperatures, especially when sample size is taken into consideration (Table IV). At 15 K, the sample size was 50 μL, at 77 K it was 200 μL, and at 298 K a 1 mL sample size was assumed. The LOD was calculated as that amount of sample giving a signal equal to three times the standard deviation of the blank signal. The standard deviation of the blank was taken as one-fifth of the peak-to-peak noise in the blank spectrum. Table V shows that the linear dynamic range (LDR) is between two and three orders of magnitude in all cases. The low concentration limit of the LDR was taken as the LOD. The upper limit was taken as that concentration where the signal falls 10% below the extrapolated linear region of the calibration curve. The slope of the log-log calibration curve is a good measure of the linearity of the data. A perfect line gives a slope of one. Each curve has a slope of unity to at least two significant figures. The reproducibility of the measurements is

Table II. Full-widths at Half Maximum (FWHM) for the Emission Bands of PAHs in Hexane Matrices at Low Temperature.

PAH	Emission Wavelength (nm)	Observed FWHM (15 K) (nm)	Literature FWHM (77 K)[a] (nm)
Anthracene	383.2	0.5	0.9
1,12-Benzoperylene	405.9	0.4	0.9
1,2-Benzopyrene	537.3	0.4	1.2
3,4-Benzopyrene	402.4	1.4	0.5
Coronene	563.8	0.8	-
Naphthacene	480.0	1.7	1.9
Naphthalene	322.0	1.7	2.3
Perylene	452.8	0.4	0.9
Phenanthrene	458.6	0.4	1.2
Pyrene	371.4	0.4	0.7
Triphenylene	461.6	0.7	-

[a] Taken from reference 31.

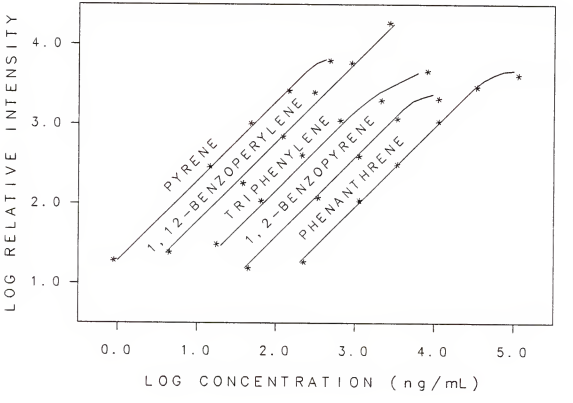

Figure 16. Low temperature analytical calibration curves.

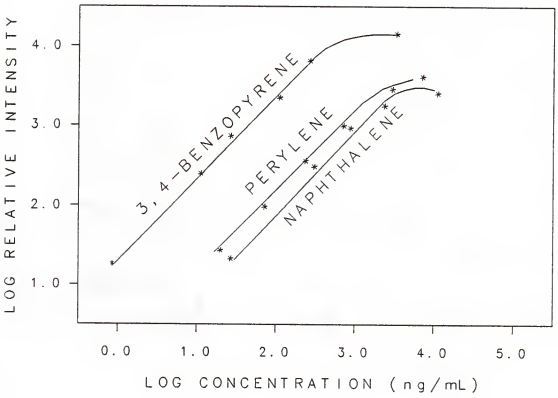

Figure 17. Low temperature analytical calibration curves.

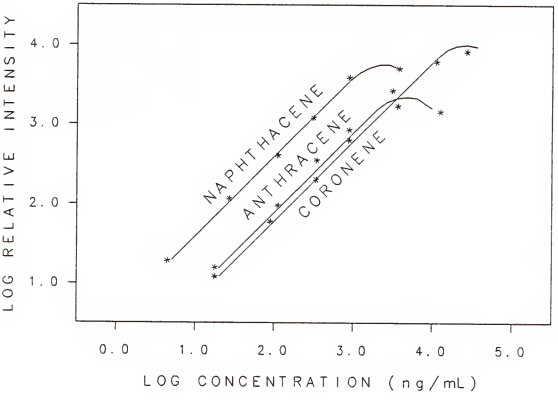

Figure 18. Low temperature analytical calibration curves.

Table III. Limits of Detection for PAHs in Hexane at Three Different Temperatures.

PAH	Excitation Wavelength (nm)	Emission Wavelength (nm)	LOD (ng/mL) 15 K	77 K[a]	298 K[b]
Anthracene	358	377.9	20	20	0.002
1,12-Benzoperylene	386	405.9	4	7	0.04
1,2-Benzopyrene	333	537.3	40	20	0.07
3,4-Benzopyrene	386	402.4	0.9	1	0.008
Coronene	337	563.8	20	-	0.03
Naphthacene	447	480.0	5	7	0.001
Naphthalene	278	322.0	30	40	0.2
Perylene	419	474.6	20	9	0.001
Phenanthrene	296	458.6	200	20	0.07
Pyrene	337	371.4	1	8	0.04
Triphenylene	288	461.6	20	-	0.04

[a] Taken from reference 31.
[b] Taken from reference 70.

Table IV. Absolute Limits of Detection for PAHs in Hexane at Three Different Temperatures.

PAH	Excitation Wavelength (nm)	Emission Wavelength (nm)	LOD (ng) 15 K	77 K[a]	298 K[b]
Anthracene	358	377.9	1	4	0.002
1,12-Benzoperylene	386	405.9	0.2	1	0.04
1,2-Benzopyrene	333	537.3	2	4	0.07
3,4-Benzopyrene	386	402.4	0.05	0.2	0.008
Coronene	337	563.8	1	-	0.03
Naphthacene	447	480.0	0.3	1	0.001
Naphthalene	278	322.0	2	8	0.2
Perylene	419	474.6	1	2	0.001
Phenanthrene	296	458.6	10	4	0.07
Pyrene	337	371.4	0.05	2	0.04
Triphenylene	288	461.6	1	-	0.04

[a] Taken from reference 31.
[b] Taken from reference 70.

Table V. Calibration Curve Data for PAHs in Hexane at 15 K.

PAH	Slope of Log-Log Plot	Linear Dynamic Range (orders of magnitude)	RSD (%)
Anthracene	1.039	2.1	7.8
1,12-Benzoperylene	1.002	2.4	6.1
1,2-Benzopyrene	1.001	2.2	10.7
3,4-Benzopyrene	1.009	3.0	8.8
Coronene	0.996	3.0	7.6
Naphthacene	1.006	2.5	4.6
Naphthalene	1.024	2.0	3.9
Perylene	1.009	2.0	4.0
Phenanthrene	1.008	2.5	3.6
Pyrene	1.020	2.4	8.9
Triphenylene	0.999	2.5	8.2

given as the relative standard deviation found for four measurements (four sample injections).

Six-component mixture. To further demonstrate the usefulness of the rotatable cooling device, a synthetic six-component PAH mixture was prepared in hexane. After injection, the concentration of each component was found by comparing the signal observed with the appropriate analytical calibration curve (Table VI). Four injections were made and the mean concentration was determined. Confidence limits are stated for a 90% level of probability. The percent error in each case is below 40%. The error is attributed to several factors: overlap of peaks, poor reproducibility of injections, prefilter and post-filter effects, and the possible formation of excimers. Nevertheless, such error is tolerable in many cases especially when multicomponent analysis can be performed rapidly.

The results from this experiment reveal that the moving sample cooling device is capable of cooling samples to 15 K rapidly and reliably. The spectral quality observed compares favorably with those given in the literature at 63 K (27) and at 15 K (69). Also demonstrated is the usefulness of the device in the analysis of multicomponent synthetic PAH mixtures. In the next chapter, this property will be applied to the rapid determination of several PAHs in cooked beef.

Table VI. Quantitative Analysis of a Six-component Mixture of PAHs in Hexane.

PAH	Concentration Found (mg/L)	Actual Concentration (mg/L)	Percent Error
1,12-Benzoperylene	0.24 ± 0.14	0.30	20
1,2-Benzopyrene	0.40 ± 0.15	0.30	33
Coronene	0.42 ± 0.20	0.36	17
Perylene	0.41 ± 0.14	0.30	37
Phenanthrene	4.60 ± 2.82	3.30	39
Triphenylene	0.60 ± 0.45	0.70	14

CHAPTER 4

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN COOKED BEEF

Introduction

The existence of several PAHs in foods and food additives is well documented (58,59). Several PAHs have been identified at the part-per-billion level in some uncooked market basket commodities including vegetables, beverages, fats and oils, dairy products and meats (71). Similar levels have also been reported on the surface and in the interior of fruits such as Valencia oranges (72). The trace contamination in these fresh foods is a direct result of man's pollution of the air and water systems serving the farmlands where these foods are produced. More often, however, PAHs are introduced into our foods through the cooking process. At least twenty-five different aromatic compounds have been identified in curing smoke, and of these, seven have been identified at the part-per-billion level in smoked foods (60). The amount of 3,4-benzopyrene in the skin of smoked meats has been shown to be at least ten times higher than the concentration in the interior (73). A general method for the extraction and estimation of polycyclic aromatic hydrocarbons in smoked foods was introduced by Howard and coworkers in 1966 (74). They also introduced an extraction and estimation procedure specific for 3,4-benzopyrene (75). This compound has often been used as an

arbitrary indicator of the presence of carcinogenic PAHs in smoked and charcoal grilled meats (76,77). A later, more extensive study identified twenty-two different PAHs in frankfurters cooked by six different grilling techniques (78). Of these techniques, grilling over various fires was shown to produce substantially higher levels of PAHs in the meat than did electric grilling. The PAHs in grilled meats are believed to arise from the pyrolysis of fat (79). Melted fat from the heated meat drips onto the hot coals and is pyrolyzed, giving rise to PAHs which are deposited onto the meat surface as the smoke rises (78).

Grilled meats, especially hamburgers, make up a significant fraction of the American diet so identification of contaminants in them is of great importance. Since millions of hamburgers are sold each year in this country's restaurants, and millions more are grilled in private homes, it is important to identify the PAHs that they contain, to determine the levels at which they occur, and to estimate the health risks that they impose on the population. This chapter concentrates on the identification and quantification of PAHs in fried hamburger, charcoal grilled hamburgers, and the hamburgers sold by four major fast-food restaurants. In the current study, the moving sample cooling device is used in the analysis of broiled hamburger extracts, and the results are compared to those obtained by two other methods often used for the qualitative analysis of complex mixtures: constant energy synchronous luminescence spectrometry (CESLS) and high performance liquid chromatography (HPLC) (54, 80-83).

Experimental

Chemicals. The compounds 1,12-benzoperylene, 3,4-benzopyrene and pyrene were obtained from Aldrich (Milwaukee, WI). Each had a purity of 98% or better and was used without further purification. Perylene was obtained from Fluka (Ronkonkoma, NY) at 98% purity and was used as received. Spectroscopic grade n-hexane and methanol were obtained from Burdick and Jackson (Muskegon, MI). Reagent grade cyclohexane, potassium hydroxide and N,N-dimethyl-formamide (DMF) were obtained from Fisher (Fair Lawn, NJ). Standards of each PAH were prepared in n-hexane in concentrations ranging from 1-10,000 ng/mL. Synthetic mixtures of the four PAHs were prepared at concentrations similar to those found in the hamburger extracts.

Cooking procedure. Four ground beef samples were purchased at a local supermarket. Each sample was purchased on a different day and each was labelled "30% fat or less". The hamburger was shaped into 1/4 lb patties before cooking.

The grilling procedure was similar to that used by Doremire et al. (77). Patties were cooked on a small Hibachi grill 10 cm above the coals. The patties were cooked 5 minutes on each side to a "well-done" state. The cooked weight of each hamburger was between 60 and 70 g.

Several hamburger patties were fried in an electric frying pan to serve as reference samples. Some were spiked with known amounts of pyrene after cooking to determine the percent recovery of the extraction procedure. In this case, 5 mL of a pyrene standard solution was added to 100 g of the ground hamburger prior to extraction.

Extraction procedure. The extraction method was based on work done by Grimmer and Bohnke (65,84). Their extraction method for PAHs in meats has been used by several workers since then (77,78,85) and has been adopted by IUPAC as a "recommended method for the determination of PAHs in foods" (86). One minor change was made to the procedure. In the final step, n-hexane, a Shpol'skii solvent, replaced the more conventional cyclohexane solvent. A brief summary of the adopted procedure follows (see reference 84 for a detailed description).

The cooked beef sample was ground in a meat grinder. A 150 g sample was transferred to a round bottom flask where it was boiled in 300 mL aqueous-methanolic 2 M KOH (methanol:water, 9:1) for four hours. The saponified sample was then extracted twice with 800 mL cyclohexane. The combined cyclohexane layers were evaporated to 100 mL on a rotary evaporator and then extracted twice with 100 mL DMF:water (9:1). Finally, the DMF layer was extracted twice with 100 mL n-hexane, and the combined n-hexane layers were evaporated to 10 mL on a rotary evaporator, quantitatively transferred to a 25 mL volumetric flask, and diluted to the mark with additional n-hexane. The entire extraction procedure required approximately eight hours for completion. No further sample preparation steps were taken.

Low temperature molecular luminescence. The spectrometric system used for obtaining low temperature fluorescence spectra for each sample has been previously described in detail (67). Aliquots of 50 μL of hamburger extract were injected through a septum onto the moving brass belt in contact with the cold tip. While the sample was on the cold stage, low temperature fluorescence spectra were taken

for each PAH identified. After obtaining reproducible spectra, the brass belt was moved so that the sample was no longer in contact with the cold head, and as the belt warmed, the sample evaporated into the vacuum chamber. Thus, the cooling device was self-cleaning. Additional samples could be injected immediately without breaking the vacuum or warming the refrigerator. The entire procedure required less that 5 minutes per sample. All samples were injected four times.

Constant energy synchronous luminescence. The experimental set-up used to take CESLS spectra was the same one used in past publications (54,81,82). The spectral bandpass for the 0.33 m monochromators (Heath) was 0.4 nm. Scan rates were approximately 50 nm/minute, providing constant energy scans with an excitation range from 200-500 nm in six minutes. A constant energy difference ($\Delta\bar{\nu}$) of 3500 cm^{-1} was chosen because all four of the PAHs determined in cooked beef appear in such spectra. About 0.5 mL of the hamburger extract was transferred into a long quartz cell. The cell was immersed in a liquid nitrogen filled Dewar flask positioned in the sample compartment of the spectrometric system. Sampling time including manipulation of the Dewar flask and clean-up of the quartz cell was about 15 minutes.

Liquid chromatography. The chromatographic set-up and detection system were the same as those reported earlier (83). PAHs were separated on a 4.6 mm i.d. x 250 mm long column (Beckman) with 5 μm ODS packing material. The mobile phase was acetonitrile/water, 80/20 (V/V), and the flow rate was 1 mL/min. Injection volumes were 10 μL at 25°C. Three detection modes were employed: a 254 nm ultraviolet

absorption detector, a fluorescence detector with λ_{ex} = 380 nm and λ_{em} = 440 nm, and a second fluorescence detector with λ_{ex} = 340 nm and λ_{em} = 400 nm. Chromatographic time for a single injection was about thirty minutes.

Results and Discussion

Extraction efficiency. Table VII shows the recovery of pyrene added to cooked hamburger. A 5 mL aliquot of a standard pyrene solution (860 ng/mL) was added to each of three 100 g fried hamburger samples. Percent recovery in each case was around 70%, which agrees well with previous results found in the literature (84). Similar percent recoveries have been reported for perylene, 1,12-benzoperylene, and 3,4-benzopyrene (84). In all cases, 75-85% of the added compound was recovered in the extraction procedures.

Shpol'skii spectra. Some examples of low temperature fluorescence spectra of the grilled beef extracts are given in Figures 19-22. The particular sample shown is one that was charcoal grilled in the laboratory. Most of the fluorescence peaks in Figure 19 can be attributed to pyrene. The compounds giving rise to the two small peaks at 374 nm were not identified. Similarly, the majority of the fluorescence peaks in Figure 20 can be attributed to 1,12-benzoperylene. All of the remaining peaks are attributed to 3,4-benzopyrene (Figure 21). Figure 22 shows the small amount of perylene found in the grilled beef extracts. These figures demonstrate that the high resolution achieved via the Shpol'skii effect makes possible the spectrometric identification of compounds whose emission spectra usually overlap.

Table VII. Extraction Recovery of Pyrene Added to Cooked Beef.

Sample	Replicate	Amount added (ppb)	Amount found (ppb)	Percent recovered
Current Results	1	43	30	70
	2	43	32	74
	3	43	30	70
Previous Results[a]	1	17	12	71
	2	17	12	71
	3	17	13	76

[a] See reference 84.

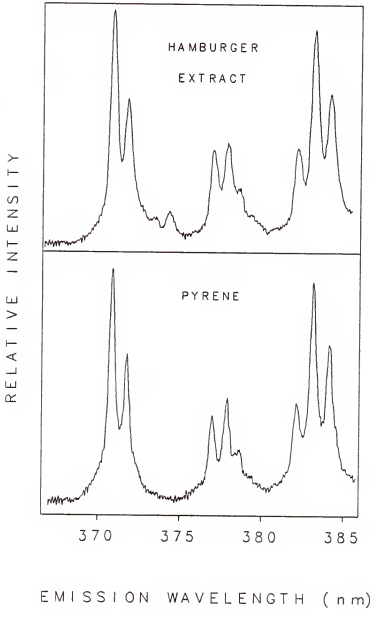

Figure 19. Identification of pyrene in a grilled beef extract by low temperature molecular luminescence spectrometry. The excitation wavelength is 337 nm.

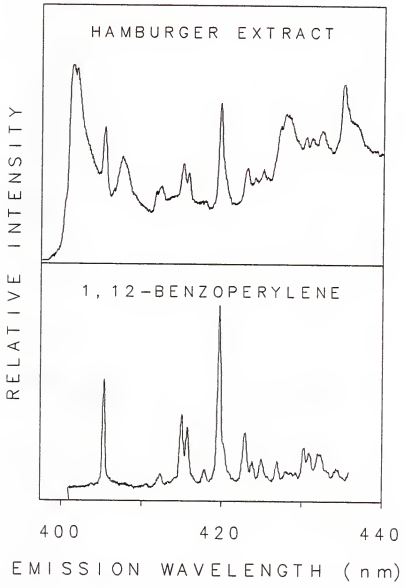

Figure 20. Identification of 1,12-benzoperylene in a grilled beef extract by low temperature molecular luminescence spectrometry. The excitation wavelength is 386 nm.

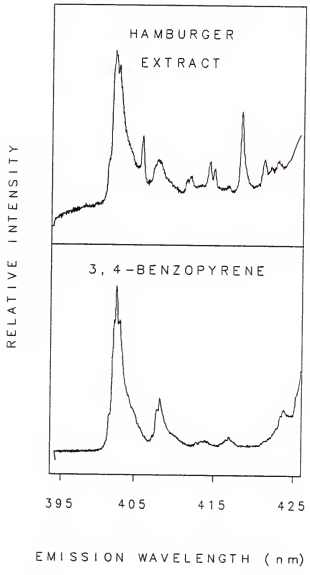

Figure 21. Identification of 3,4-benzopyrene in a grilled beef extract by low temperature molecular luminescence spectrometry. The excitation wavelength is 386 nm.

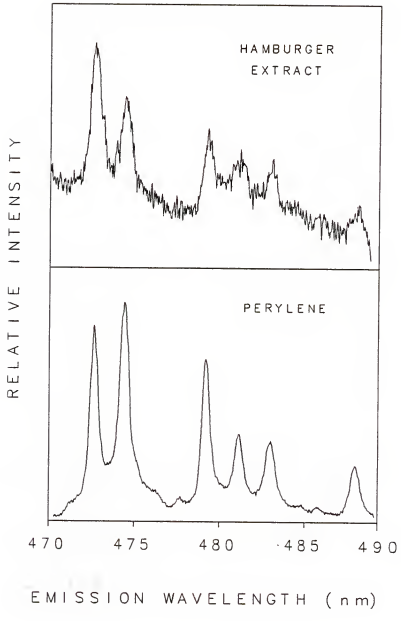

Figure 22. Identification of perylene in a grilled beef extract by low temperature molecular luminescence spectrometry. The excitation wavelength is 420 nm.

Constant energy scans. Figure 23 is a CESLS scan of the same hamburger extract shown in Figures 19-22. Notice that all four PAHs are represented in a single scan. As a result, analysis time is decreased, but the identification of particular compounds becomes more difficult since the spectrum is more complex than those obtained at 15 K. Further resolving power is lost since each PAH has only 1-3 characteristic emission peaks in the CESLS scan, whereas they have 6-10 in the low temperature scans. Another drawback of CESLS is the loss in sensitivity due to the restrictions imposed on the excitation and emission wavelengths. Since a constant energy difference must be maintained between the two, it is highly unlikely that the maximum excitation and emission wavelengths will occur simultaneously for more than one compound in a single CESLS scan. For instance, the fluorescence peaks due to perylene in Figure 23 occur at an excitation wavelength of about 380 nm, but the maximum fluorescence excitation wavelength for perylene occurs at 419 nm. In a CESLS scan with $\Delta\bar{\nu} = 3500\ cm^{-1}$, when the excitation monochromator is at 419 nm, the emission monochromator is well beyond the maximum emission wavelength for perylene and no signal is observed.

Liquid chromatography. Liquid chromatographic separation of a charcoal grilled hamburger sample is shown in Figure 24. The four PAHs detected earlier are easily separated and identified here. Notice that several other fluorescent and non-fluorescent components of the extract are also resolved. No attempt was made to identify all of these constituents. One drawback of the HPLC method is the rather long analysis time observed in this work (30 minutes). The major drawback, however, is the lack of sufficient data for the

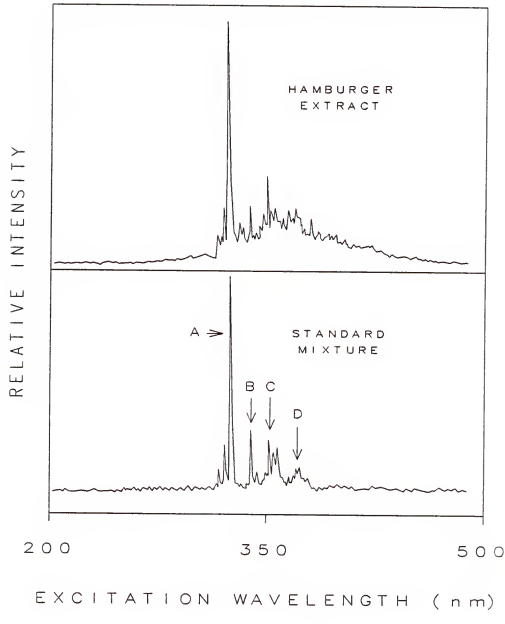

Figure 23. Constant energy synchronous luminescence spectrum of a grilled beef extract in n-hexane at 77 K. The lower spectrum is for a standard mixture of pyrene (A), 3,4-benzopyrene (B), 1,12-benzoperylene (C), and perylene (D). A constant energy difference ($\Delta\bar{\nu}$) of 3500 cm^{-1} was used in both cases.

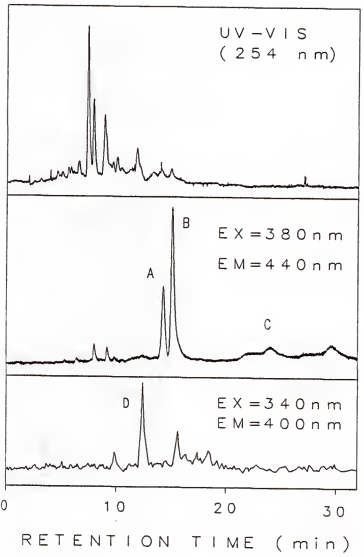

Figure 24. Liquid chromatographic separation of a grilled beef extract with three modes of detection. The labelled peaks are identified as perylene (A), 3,4-benzopyrene (B), 1,12-benzoperylene (C), and pyrene (D).

identification of individual components of the mixture. Multiple detector modes make this task easier as can be seen in Figure 24.

Quantitative analysis. Table VIII shows the amounts of PAHs found in each of the cooked beef samples tested. All concentrations were found for the low temperature molecular luminescence spectra, by comparing peak heights to the calibration curves reported earlier (67). Relative standard deviations in each measurement were better than 10%. Deviations in the PAH content of hamburgers grilled on the four different days were high. However, the levels of PAHs in charcoal grilled meat have been shown to be greatly dependent on the fat content of the beef and the temperature of the coals (79). Nevertheless, the amounts of PAHs found in the grilled beef samples agreed well with those found by other workers (77). Only one of the fast-food restaurants sold hamburgers with measurable PAH content. This particular restaurant is known to flame broil their beef, while all of the other restaurants fried their hamburgers.

Conclusions. Table IX compares the three methods used for the determination of PAHs in complex real samples such as cooked beef. While HPLC is the least expensive and easiest to operate, it also yields the smallest amount of data resulting in lower identification power. Low temperature molecular luminescence spectrometry (LT-MLS) is the fastest technique and it gives more spectral information than the other two. CESLS is a "middle of the road" technique that offers no real advantages over LT-MLS other than cost of equipment.

Table VIII. PAHs Found in Cooked Hamburgers.

Sample	Source	Amount Found (ppb)			
		Pyrene	3,4-Benzo-pyrene	Perylene	1,12-Benzo-perylene
Charcoal-Grilled Beef	1	25	20	8	21
	2	52	34	9	28
	3	2	9	3	6
	4	29	13	4	9
	5[a]	18	8	2	5
	6[b]	NR[c]	22	NR	NR
Fried Beef		ND[d]	ND	ND	ND
Fast Food Restaurants	1	18	7	3	7
	2-4	ND	ND	ND	ND

[a] See reference 52.
[b] See reference 77.
[c] NR = not reported.
[d] ND = not detected.

Table IX. Comparison of Three Methods for the Determination of PAHs in Cooked Beef.

Method	Sensitivity (LOD in ppb 3,4-Benzo-pyrene)	Identification power (spectral features per component)	Reproducibility (% RSD for 3,4-Benzopyrene)	Analysis time[a] (min)
LT-MLS	0.9[b]	6-10	9[b]	5
CESLS	0.2[c]	1-3	6[c]	15
HPLC	1.0[d]	1	6[d]	30

[a] Analysis times for this work only.
[b] See reference 67.
[c] See reference 30.
[d] See reference 83.

CHAPTER 5

CONCLUSIONS

Summary

The work presented here has described a novel and innovative device which allows rapid changes of samples deposited onto a cryogenic cooling belt housed in a vacuum chamber. Liquid samples can be frozen, examined spectroscopically, and removed in less than five minutes. The device is easy to construct and relatively inexpensive to build. Compared to previously constructed moving sample cooling devices, the continuous cooling belt offers several distinct advantages. First, the device can accommodate samples deposited continuously or in discrete segments. Only one previously reported device is operable in both modes (41). Secondly, the device is self-cleaning, so it can be operated indefinitely without the need for refrigerator warm-up time. No similar device is self-cleaning. Finally, the device has only a limited number of small moving parts, so the entire cryostat need not be moved. This makes the cooling belt system the simplest in design of all of the previously described moving sample cooling devices.

Of the five devices described in Chapter 1, none has any advantage over the newly constructed cooling belt. This device is also easier to use than most liquid nitrogen sample cooling systems. The only real disadvantages of the present system are associated with

the Joule-Thomson refrigerator that must be employed. Inevitably, such refrigerators are large, heavy, noisy and expensive. Nevertheless, the advantages far outweigh these annoyances, especially since all of the devices discussed here employ similar refrigerators. A consolation with the moving cooling belt system is that the refrigerator is always stationary so it could easily be housed in an attractive, noise-reducing cabinet.

The work presented in Chapter 3 demonstrates that the new cooling device is effective and reliable when used for cooling samples in low temperature molecular luminescence spectrometry. Chapter 4 shows that the device works well for real applications such as the determination of PAHs in cooked beef. Obviously, this device could save time and money in many other analytical applications including spectrometric measurements involving Raman scatter, infrared absorption, ultraviolet-visible absorption and matrix isolation. The device could easily be adapted to any of these techniques.

Applications

Future work involving the continuous sample cooling device will take two directions. First, the device will be applied to the analysis of discrete sample volumes by the various spectrometric techniques mentioned above. Liquid samples will include extracts from environmental samples such as river sediment and air-borne particulates. Also, direct liquid sampling is planned for clinical and pharmaceutical fluids and for waste water analysis. The samples will be examined for PAHs, drugs and priority pollutants.

Future changes to the sample introduction port on the cooling device will accommodate direct analysis of gaseous and solid samples. The gaseous samples may require a heated inlet port and a carrier gas such as helium. The solid samples will probably require a differentially pumped vacuum chamber with a conveyor belt attachment moving from the open atmosphere to the high vacuum cooling chamber through a series of smaller chambers with intermediate pressures.

Currently, work has been focused on the analysis of continuously deposited samples such as the effluent from gas or liquid chromatography columns. Shpol'skii spectrometry has often been used in conjunction with these techniques (36, 87, 88), but in each case discrete sample volumes were collected in small segments eluting from the column. In the current work, low temperature Shpol'skii spectra will be acquired "on the fly" as a particular component elutes (Figure 25). A microbore liquid chromatography column will be used with an amino-silane packing material. The mobile phase will be n-hexane flowing at a rate of 50 μL/min or less. Complex mixtures of PAHs will be analyzed, including standard mixtures and real samples. Such a combination of separation and spectrometric methods will add higher selectivity to an already powerful analytical technique.

A low temperature matrix isolation detector for gas chromatography is planned in the near future. In this case, the effluent from a capillary column gas chromatograph will be frozen directly onto the moving band. A carrier gas of 97% helium and 3% argon will be employed. The helium gas will be pumped away, while the sample molecules are trapped in the frozen argon matrix. Detection will be accomplished by infrared absorption spectrometry.

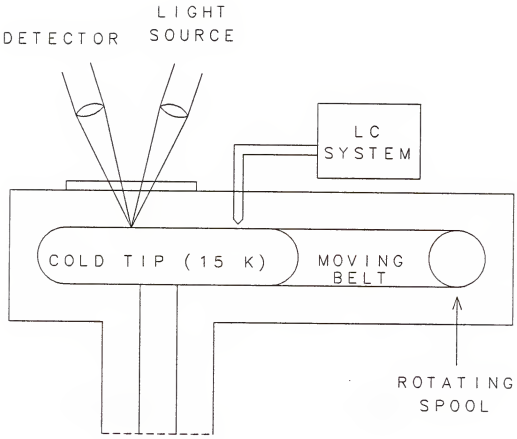

Figure 25. Simplified cross-sectional side-view of the vacuum chamber for an HPLC/LT-MLS interface.

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APPENDIX

SHPOL'SKII SPECTRA

The following is an appendix containing a library of the Shpol'skii spectra observed for the eleven polycyclic aromatic hydrocarbons used in this study. All spectra were taken with an 8 nm excitation bandwidth and a 0.4 nm emission monochromator bandpass. Fifty microliters of each PAH solution in n-hexane were injected onto the cooling belt and luminescence spectra were recorded at 15 K. The optimum excitation wavelength was determined by manually scanning the excitation monochromator until the largest luminescence intensity was observed. The type of luminescence observed (fluorescence or phosphorescence) was determined by comparison with previously published data. The wavelengths of the major luminescence peak maxima are labelled in each figure.

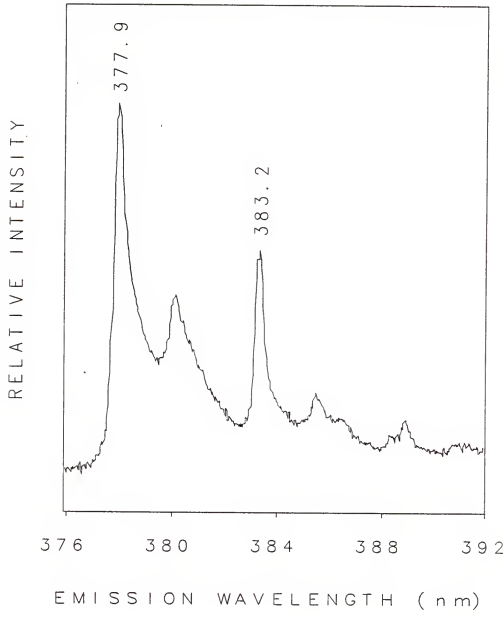

Figure 26. The fluorescence spectrum of anthracene. The concentration is 10 mg/L and the excitation wavelength is 358 nm.

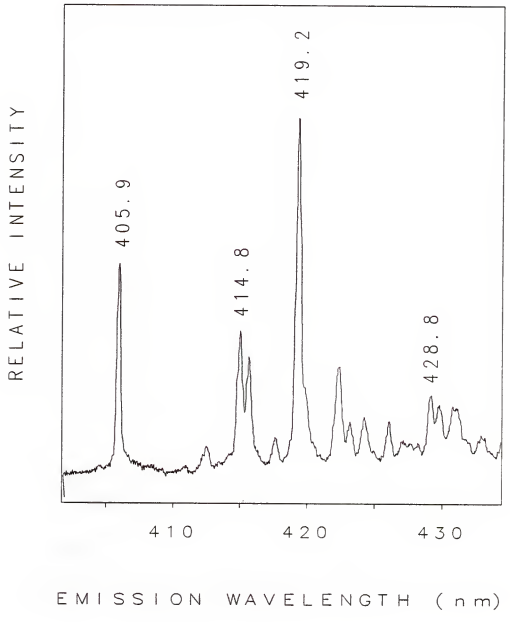

Figure 27. The fluorescence spectrum of 1,12-benzoperylene. The concentration is 0.3 mg/L and the excitation wavelength is 386 nm.

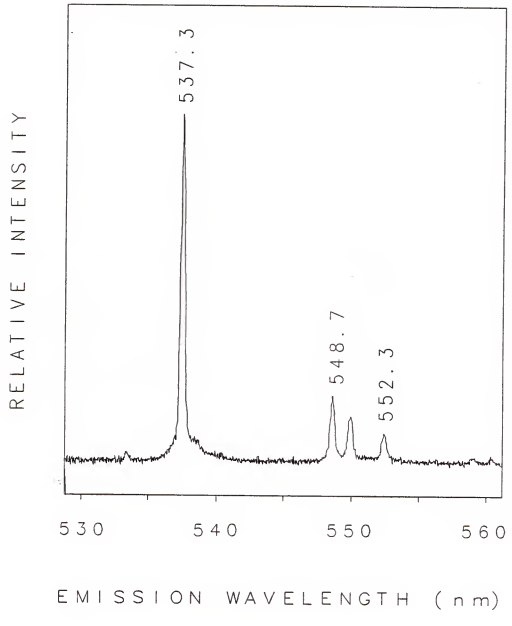

Figure 28. The phosphorescence spectrum of 1,2-benzopyrene. The concentration is 3 mg/L and the excitation wavelength is 333 nm.

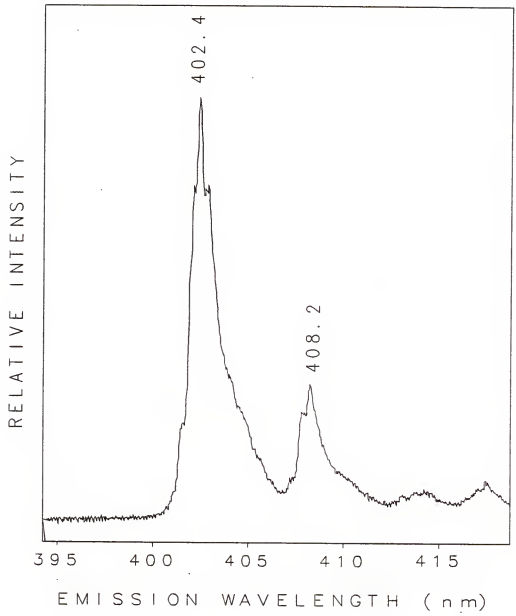

Figure 29. The fluorescence spectrum of 3,4-benzopyrene. The concentration is 0.3 mg/L and the excitation wavelength is 386 nm.

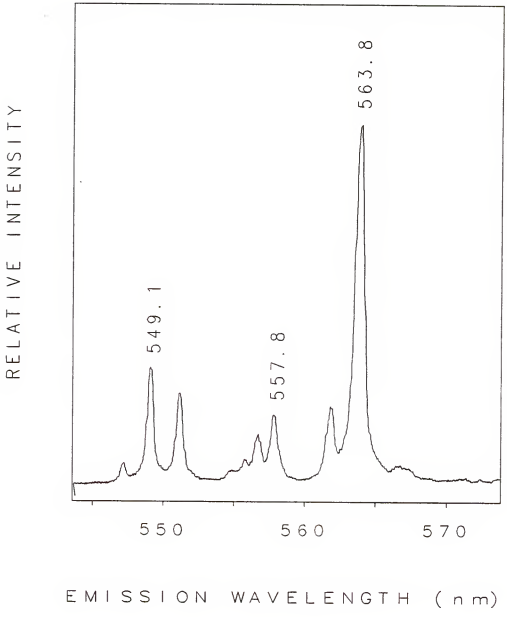

Figure 30. The phosphorescence spectrum of coronene. The concentration is 30 mg/L and the excitation wavelength is 337 nm.

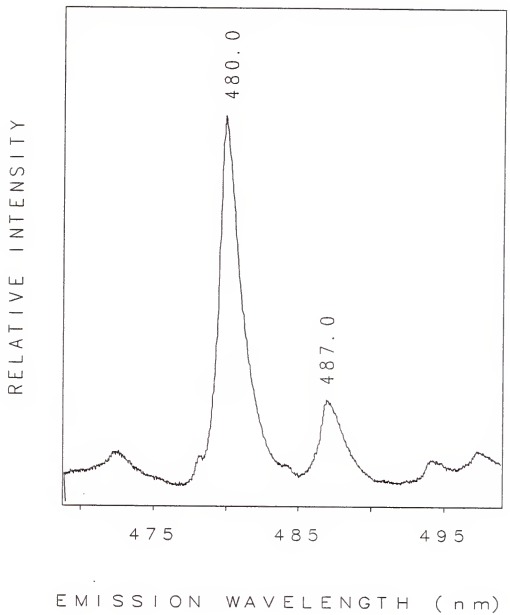

Figure 31. The fluorescence spectrum of naphthacene. The concentration is 30 mg/L and the excitation wavelength is 447 nm.

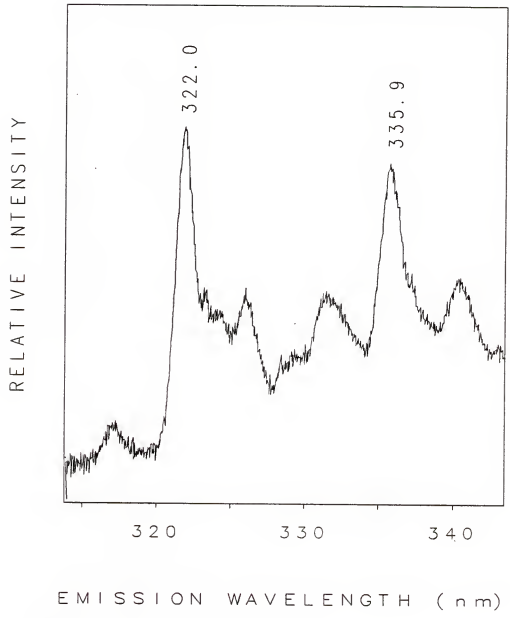

Figure 32. The fluorescence spectrum of naphthalene. The concentration is 3 mg/L and the excitation wavelength is 278 nm.

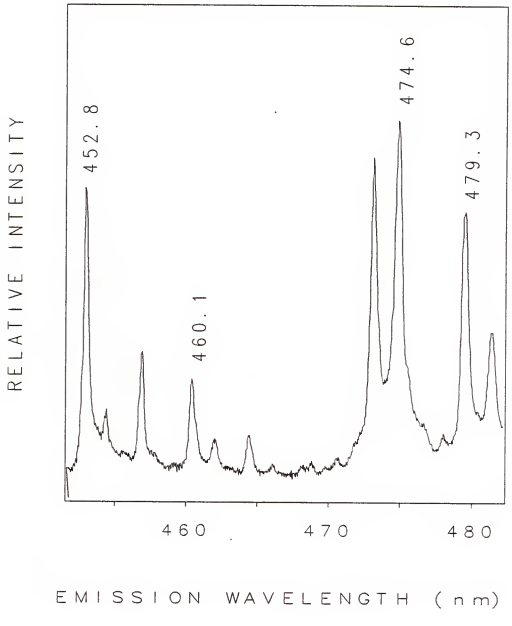

Figure 33. The fluorescence spectrum of perylene. The concentration is 0.7 mg/L and the excitation wavelength is 419 nm.

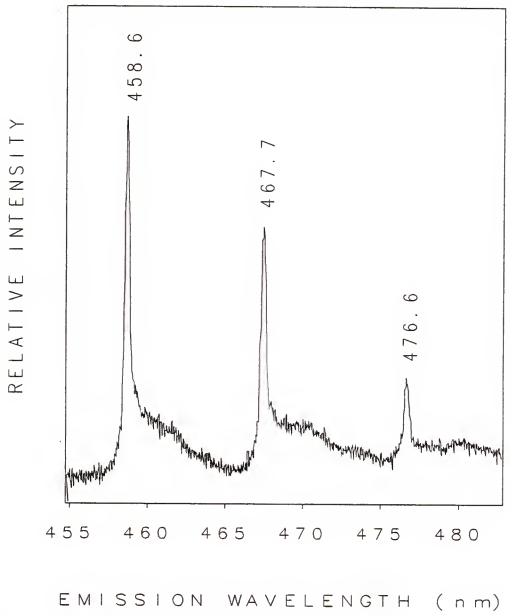

Figure 34. The phosphorescence spectrum of phenanthrene. The concentration is 30 mg/L and the excitation wavelength is 296 nm.

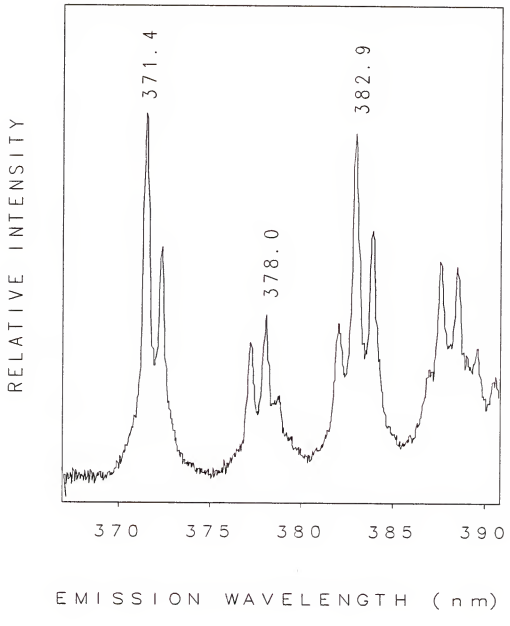

Figure 35. The fluorescence spectrum of pyrene. The concentration is 0.13 mg/L and the excitation wavelength is 337 nm.

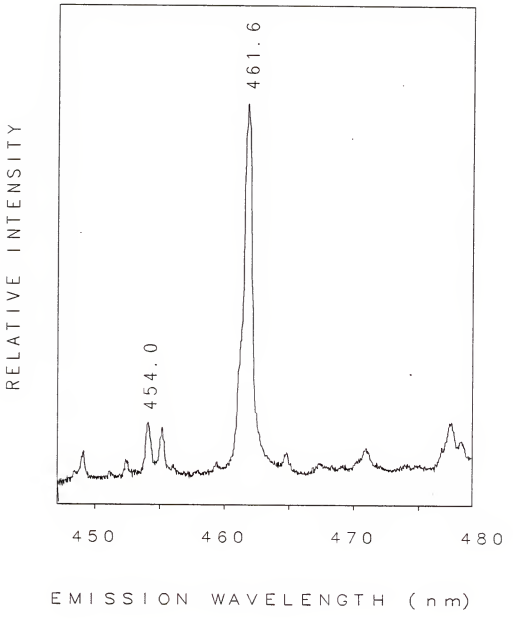

Figure 36. The phosphorescence spectrum of triphenylene. The concentration is 3 mg/L and the excitation wavelength is 288 nm.

BIOGRAPHICAL SKETCH

Bradley Todd Jones was born on July 15, 1962, in Thomasville, North Carolina. He graduated from Thomasville Senior High School in June 1980. He entered Wake Forest University in Winston-Salem, North Carolina, in August of that same year. On December 31, 1983 he married his high school sweetheart, Melanie Carol Maley. In May 1984 he received the Bachelor of Science degree with a major in chemistry and a minor in mathematics. His first research experience in analytical chemistry took place in the laboratory of Dr. Willie Hinze in the spring of 1984, where he monitored micelle-catalyzed reactions by ultraviolet absorption spectrometry. He entered the graduate school of the University of Florida in August 1984, where he later joined the research group of James D. Winefordner. Brad is currently a candidate for a Doctor of Philosophy degree in analytical chemistry.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Graduate Research Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Martin T. Vala
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Steven G. Schulman
Professor of Pharmaceutics

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John G. Dorsey
Associate Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Assistant Professor of
Chemistry

This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the Doctor of Philosophy.

April 1988

Dean, Graduate School